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Dear Roel:

Many thanks for your letter. I am very pleased to learn that you are definitely interested in joining us.

I am surprised that your contract would confine you to work on MMTV proteins; it is usual, in my experience, for funds applied at the graduate or postdoctoral level to be granted with a good deal of freedom, so long as the recipient is doing reasonable science in the intended area and in the expected laboratory. However, as you point out, I don't think there would be any problem rationalizing anything you do with us as applicable to problems of MMTV proteins.

At this stage, it might be most useful for me to outline briefly what is going on with MMTV at the moment and to indicate the general areas in which I expect things to happen. (Of course, you would not be confined to work with MMTV when you come. There is a lot going on with various avian viruses, particular ASV and leukosis viruses, and I have plans to start a small project on hepatitis virus(B type) in a year or so.)

John Majors has been cloning and sequencing MMTV DNA. Thus far he has obtained the strong stop sequence from DNA made in vitro and has made a large number of clones from closed circular MMTV DNA, using the lambda (WES.B) cloning system. His goals are to determine the nucleotides sequences of the interesting regions of MMTV DNA: the 1200 between env and the 3' end of RNA and the probably few hundred bases between the 5' terminus and gag. In addition, he has made a series of cloned lines of mink cells infected with MMTV and bearing one or two copies of DNA, some of which are deleted. He will clone some of these proviruses in lambda and will establish the sequences at the joints and in the flanking regions. The materials he generates will also be used by Keith Yamamoto for steroid binding studies and in vitro transcription and by [redacted]me for [redacted] experiments (to be started here) in which putative regulatory regions of MMTV DNA are linked to polyoma DNA.

Don Robertson is continuing to characterize intracellular RNA's of MMTV, using the "Northern" transfer procedure, UV mapping, S1 mappin, and in vitro translation. He is focussing particularly upon the events that follow steroid induction of MMTV RNA synthesis, since he has clones of mink cells in which as much as 1000 fold augmentation occurs and with sufficient rapidity to follow the processing steps.- Putative intermediates in processing have been sighted, and these will be mapped on the genome. We are hoping to find highly responsive cells with a single provirus, since this would provide the best opportunity for mapping the promoter with the UV technique and for seeking precursors by S1 mapping of nuclear RNA. Don is also extending his analysis of polyribosome associated RNA's, using the Northern transfer procedure to analyze fractions and preparing membrane bound and free polysomes. This exercise is to confirm preliminary evidence that 24S RNA is in membrane bound polys and to determine whether the 13S species is truly an mRNA (I am uncertain of its status although it appears by conventional tests to be in polysomes).

Jackie Dudley (late of Janet Butel's lab) is principally interested in the mechanism of oncogenesis by MMTV. Although she has only been in the lab for several months, she has directed attention to

ew fronts: the gene products (if any) of the 13S or any other oogenomic species other than 24S RNA, using affinity chromatography with cloned DNA to purify RNA for translation in vitro; tumor antigens, attempting to raise antisera in rabbits and other animals by injection of MMTV or MMTV infected cells than to immunoprecipitate from infected cells or translates made in vitro; and viral mutants using cloned DNA (which must first be shown to be virulent) to generate deletions and create recombinants with other viruses, all then to be tested in animals for oncogenicity.

At the moment, not much is happening in SF in relation to endogenous DNA. Craig is pursuing several aspects of that problem in New Orleans (looking at FL hybrids of lots of inbred stains, developing an MMTV-negative line from feral mice, and looking at feral mice from round the world), and Vince continues to look at the chromosomal distribution of MMTV-DNA in Ontario. (He has also recently found that the so-called MMTV-0 is not endogenous to BALB/c and is apparently acquired by (inadvertant) horizontal infection.) I would, however, expect that more will be done in SF with endogenous DNA. One specific plan is to put cloned virulent MMTV DNA into BALB/c germ lines, this to be done in collaboration with Rudi Jaenisch who will microinject eggs. The impact of this new provirus will then be evaluated by various obvious criteria. I would also like to examine more closely the host DNA immediately adjacent to endogenous MMTV DNA in both inbred and out bred mice, to confirm or deny our proposal that these proviruses have entered the germ line by independent infections. The alternative, that considerable genetic rearrangement has occurred, would be evident from sequencing at the host-viral joints, provided that the rearrangements had not occurred precisely at the joints. I am also interested in the organization of MMTV DNA at the mtv-2 locus, but I would hesitate about embarking on this cloning project (a difficult one because of the probable tandem structure of the locus) without consultation with those who have a deeper commitment to that locus. You ask about the MMTV DNA in rat DNA. We haven't done much about that, though I have(unintentionally) annealed MMTV to various digests of Fisher rat cell DNA under conventional conditions (Southern blotting) without seeing much of interest (a few very faint bands and, in one case, a probable ribosomal band). These tests should probably be repeated at some time with cloned probes, other rat DNA's and annealing conditions of reduced stringency.

As mentioned briefly above, the other things being done with MMTV is to join the interesting (3'5') regions to polyoma DNA to assess the role of these sequences upon integration, excision, transcription, and steroidal regulation. John and I are starting these experiments wit Mike Fried but there may be room for more hands if things kprove interesting.

Why don't you consider the abbve, then let lme know what sort of things you would most like to work on. It would probably be helpful fo lyou to know that Don will be around for about one more year (hence he will leave much undone), John will be in SF for about two more lyears, and Jackie for about 2 and one half lyears.

Cheers,

Harold

PS. I should have mentioned the possibility of looking directly at the "dominant" proviruses in tumor DNA (taking advantage of the clonal property of tumors), cloning such proviruses to ask about their genetic composition. We will need tumors with one rather than several dominant proviruses to do this; we have such a situation now with avian leukosis, but an appropriate tumor should not be difficult to find.